European antimicrobial resistance surveillance as part of a community strategy

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Chapter 4

Comparability of antimicrobial susceptibility test results from 22 European countries and Israel: an external quality assurance exercise of EARSS in collaboration with UK NEQAS

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Abstract

The goal of this exercise was to organize external quality assurance (QA) of antibiotic susceptibility testing for laboratories participating in EARSS and to assess the comparability of susceptibility test results across countries, and guidelines. In September 2000, UK NEQAS distributed a set of three *Streptococcus pneumoniae* strains, two *Staphylococcus aureus* strains and one *Staphylococcus haemolyticus* strain. Laboratories reported the guideline followed, the interpretation of the susceptibility test result and the MIC, if tested. In this study we considered results 'concordant' if the reported interpretation of the participating laboratory agreed with the designated interpretation of reference laboratories. Overall, 433 (92%) of 471 laboratories from 23 countries reported back. Of the 8685 tests that were assessed, 8322 (96%) were interpreted correctly by the participants. Concordance for detection of penicillin non-susceptibility in the three *S. pneumoniae* strains was 96%, 90% and 87%, respectively. Laboratories performed extremely well in detecting oxacillin resistance in the homogeneously methicillin-resistant *S. aureus* (MRSA) strain, but the concordance rate dropped from 100% to 77% in the heterogeneously resistant MRSA strain. Concordance for detection of teicoplanin resistance in the *S. haemolyticus* strain was 82%. We stratified concordance rates first for country and then for guideline used, but observed only minor differences among countries and guidelines. Quantitative methods yielding an MIC were more concordant than non-MIC methods for penicillin resistance in the *S. pneumoniae* strains (94% versus 79%). The NCCLS guideline was the most frequently followed, by 61% of laboratories from 19 countries. This exercise shows that, overall, countries participating in EARSS are capable of delivering susceptibility data of good quality.

The comparability of susceptibility data for penicillin resistance in *S. pneumoniae* and for homogeneous methicillin resistance in *S. aureus* is satisfactory among European countries and across guidelines. However, we emphasize the importance of determining an MIC for suspected penicillin non-susceptible *S. pneumoniae* and for suspected glycopeptide non-susceptible *S. aureus*. Laboratories, particularly in some countries, may need to improve their capability to detect oxacillin resistance in heterogeneously resistant MRSA. For continuous external quality assessment we recommend that laboratories participate in national and international schemes with frequent distribution of control strains.

Introduction

Since 1999, the European Antimicrobial Resistance Surveillance System (EARSS) has been monitoring antimicrobial resistance in an increasing number of European countries. Funded by the European Commission, EARSS is an international network of national surveillance systems aiming at collecting comparable and valid resistance data. The purpose of EARSS is to document variations in antimicrobial resistance over time and place, to provide the basis for policy decisions and assess the effectiveness of interventions. EARSS is an ongoing system monitoring resistance of invasive infections of *Streptococcus pneumoniae* and *Staphylococcus aureus*. Since 2001, invasive isolates of *Escherichia coli* and enterococci have also been under surveillance, and a similar external quality assurance exercise for these pathogens was organized in September 2001. Summary results from the 2001 quality assurance (QA) exercise as well as the EARSS database are accessible through the EARSS web site (www.earss.rivm.nl).

Antibiotic susceptibility of clinical isolates of bacteria is usually tested as part of routine laboratory investigations to establish the most adequate therapy for an infection. Detection of resistance relies on specimen collection from the patient, isolation, identification and susceptibility testing of the bacterial pathogen. Only recently a reference method for the determination of minimum inhibitory concentrations (MIC) has been proposed by the European Committee for Antimicrobial Susceptibility Testing (EUCAST),[1] but there is still no European agreement on breakpoint criteria for interpreting the results into clinical categories [susceptible (S), intermediate (I), or resistant (R)]. As a result, methods for most agents still differ between countries, and interpretation of test results may differ.

The goal of this exercise was to organize external quality assurance of antibiotic susceptibility testing for laboratories participating in EARSS and to assess the comparability of susceptibility test results, as collected according to the EARSS protocol[2] across countries and guidelines. Furthermore, this exercise assessed the comparability of MIC-yielding methods versus non-MIC-yielding methods (e.g. agar diffusion tests), and provided an overview of the frequency of use of various guidelines among EARSS laboratories. Quality assessment is essential in order for EARSS to assess the validity of comparing *S. pneumoniae* and *S. aureus* susceptibility data from a large number of laboratories from numerous countries and pooling it into a European database.

Materials and methods

A set of six strains (three *S. pneumoniae*, two *S. aureus* and one *Staphylococcus haemolyticus*) was provided by the 'French Reference Center for Antibiotics–Institut Pasteur'. The strains were characterized and tested by three reference laboratories: one in France, one in Italy, and one in The Netherlands. MICs were determined by an agar dilution method in two laboratories and by Etest in the third. Each reference laboratory

interpreted the results according to its own breakpoint criteria, respectively: Comité de l'Antibiogramme de la Société Française de Microbiologie (CA-SFM), National Committee for Clinical Laboratory Standards (NCCLS) and the Commissie Richtlijnen Gevoeligheidsbepalingen (CRG). A 'designated interpretation' and a 'reference MIC' was determined for every organism–antimicrobial combination. In cases where there were differences in MIC between reference laboratories of more than one dilution step, strains were tested repeatedly until agreeing on a reference MIC or accepting a narrow 'MIC range of reference laboratories'.

The *S. pneumoniae* strains UA1283 and UA347 were intermediately resistant to penicillin G, and *S. pneumoniae* strain UA1449 was fully penicillin resistant. *S. aureus* strain UA1432 was homogeneously resistant to methicillin, and strain UA1450 was a heterogeneously resistant MRSA strain. The phenotypic expression of methicillin resistance of the *S. aureus* strains was analysed by performing two independent population analyses on agar plates containing different concentrations of the antibiotic, as described by Tomasz et al.[3] The *S. haemolyticus* strain UA1434 was resistant to teicoplanin. The United Kingdom National External Quality Assurance Scheme (UK NEQAS) reference laboratory at the Central Public Health Laboratory, Colindale, London, organized the logistics of this study and arranged the shipment of the strains. The strains were prepared as freeze-dried cultures and sent by air-freight to EARSS national co-ordinating centres in 23 countries, who distributed the strains to the 471 laboratories participating in EARSS. Laboratories were asked to identify the control strains and to test them for susceptibility to specified antimicrobials using their routine procedures (for invasive specimens). They were asked to report the clinical categorization (S, I or R) and the MIC, if performed, as well as the breakpoints and guideline(s) followed. Five weeks were allowed for return of the results to UK NEQAS. Immediately after the closing date for return of results, brief details of the intended results were posted to participant laboratories, sent by e-mail to participants with e-mail addresses, and made available on the UK NEQAS web site. Laboratories received their individual results and a summary of the aggregate results.[4] Where 10 or more laboratories within a country participated, tables of coded results specific to the country were produced.

Analysis comprised three parts: bacterial identification, antimicrobial susceptibility test results and the use of guidelines. We assessed participants' results as being concordant or discrepant with the designated interpretation where all three reference laboratories agreed on the interpretation (S or I/R), and where the range of MICs of the reference laboratories allowed unambiguous interpretation by different guidelines.
For assessing concordance we used only two categories: susceptible (S) versus non-susceptible [i.e. intermediate plus resistant (I/R)]. Results were assessed for correct

interpretation of susceptibility/non-susceptibility for oxacillin, penicillin G and erythromycin against *S. pneumoniae*; for oxacillin, methicillin, gentamicin, vancomycin, teicoplanin and erythromycin against the *S. aureus* strains; and for gentamicin, vancomycin, teicoplanin and erythromycin against the *S. haemolyticus* strain.

In this study we considered results 'concordant' if the reported interpretation of the participating laboratory agreed with the designated interpretation of the reference laboratories. The term 'concordance rate' denotes the proportion of susceptibility tests with a correct result. For each country–except for France, Hungary and Malta, with only one laboratory participating–we calculated the average concordance of participating laboratories. We also calculated for every guideline the average of the concordance of laboratories following that guideline, using Microsoft Excel (Microsoft Corporation, Release 97 SR-2; Redmond, WA, USA). We used SAS software (SAS Institute Inc., Release 8.01; Cary, NC, USA) for the calculation of the confidence intervals (CI), weighting the results for the number of tests performed in each country and considering that observations within one country are not independent.

Results

The 'designated interpretations' and MIC reference values for the strains investigated are listed in Table 4.1. Overall, 433 (92%) of 471 laboratories from 23 countries reported results (Table 4.2). Analysis of results at a national level from countries where only one laboratory participated (France, Hungary and Malta) are not presented, for confidentiality reasons and also because the results from one laboratory may not be a true representation of national performance.

Bacterial identification

Strains were identified at the genus and species level. The three *S. pneumoniae* strains were correctly identified by: 425/428 (99%), 421/425 (99%) and 413/419 (99%) of the participating laboratories. Twelve laboratories from different countries did not identify one of the three *S. pneumoniae* strains correctly at species level and one laboratory failed to identify the genus correctly. Four laboratories identified one of the strains as Streptococcus mitis, four as *Streptococcus viridans*, two as *Streptococcus sanguis*, one as *Streptococcus oralis*, one as *Streptococcus* sp., and one as *Aerococcus* sp.

The two *S. aureus* strains were correctly identified by: 422/427 (99%) and 422/423 (100%) of the laboratories. Three laboratories identified one of the strains as coagulase-negative staphylococci, two as *S. haemolyticus* and one as Staphylococcus intermedius.

The *S. haemolyticus* strain was identified by 364/424 (86%) of the laboratories as *S. haemolyticus* or coagulase-negative staphylococcus and by 46/424 (11%) of the laboratories as staphylococcus species other than *S. aureus*. Thirteen laboratories (3%) misidentified the strain as *S. aureus* and one laboratory misidentified it as *Enterococcus faecalis*.

Table 4.1. The 'designated interpretation' and 'reference MIC' or 'MIC range of reference laboratories' for every organism–antimicrobial combination that was assessed

	Designated interpretation	Reference MIC or MIC range (mg/L)
Strain UA1449, *S. pneumoniae*		
oxacillin	R	16
penicillin G	R	3–4
erythromycin	R	>256
Strain UA1283, *S. pneumoniae*		
oxacillin	R	2
penicillin G	I	0.25–0.5
erythromycin	R	>256
Strain UA347, *S. pneumoniae*		
oxacillin	R	4
penicillin G	I	0.5
erythromycin	R	>256
Strain UA1432, *S. aureus*		
oxacillin	R	>256
methicillin	R	>256
gentamicin	R	64–128
vancomycin	S	2
erythromycin	R	>256
Strain UA1434, *S. haemolyticus*		
gentamicin	R	64
vancomycin	S	2–4
teicoplanin	R	32–64
erythromycin	R	64–256
Strain UA1450, *S. aureus*		
oxacillin	R	8–64
methicillin	R	32–64
gentamicin	S	0.12–0.25
vancomycin	S	1
teicoplanin	S	0.5–1
erythromycin	S	0.25

Table 4.2. Proportion of participants returning reports specified per country

Country	Number of QA samples sent	Number of returning reports (%)	Country	Number of QA samples sent	Number of returning reports (%)
Austria	11	10 (91)	Israel	3	3 (100)
Belgium	59	57 (97)	Italy	63	53 (84)
Bulgaria	23	20 (87)	Luxembourg	5	5 (100)
Czech Republic	34	33 (97)	Malta	1	1 (100)
Denmark	5	5 (100)	Netherlands	27	25 (93)
Germany	35	31 (89)	Poland	20	19 (95)
Finland	29	25 (86)	Portugal	20	16 (80)
France	1	1 (100)	Slovenia	10	10 (100)
Greece	18	17 (94)	Spain	32	31 (97)
Hungary	1	1 (100)	Sweden	26	25 (96)
Iceland	3	3 (100)	UK	25	24 (96)
Ireland	20	18 (90)	**Total**	**471**	**433 (92)**

QA, quality assurance

Antimicrobial susceptibility testing

Of the 8685 tests that were reported and assessed in this exercise, 8322 (96%) were interpreted correctly by the participants. The average of the concordance of all antimicrobial test results across countries surpassed 90% in all control strains (Figure 4.1). This figure shows for every control strain the average and the range across countries of the concordance of all antimicrobial test results that were assessed. The lower end of the ranges in the *S. pneumoniae* strains varied between 90% and 72%. In the *S. aureus* strains the lower ends ranged between 96% and 82%. We found similar results after stratification for guidelines (Figure 4.2), with the lowest concordance rate of 67% for one guideline for strain UA347.

Oxacillin, penicillin G and erythromycin susceptibility in S. pneumoniae. The overall concordance rate to detect penicillin non-susceptibility with an oxacillin screen disc was 97%, and ranged from 96% to 99% for the three strains tested (Table 4.3). The performance of most countries was excellent, although the group of Greek laboratories showed a lower concordance rate. Ninetyseven per cent of the participants used an oxacillin disc loaded with 1 µg.

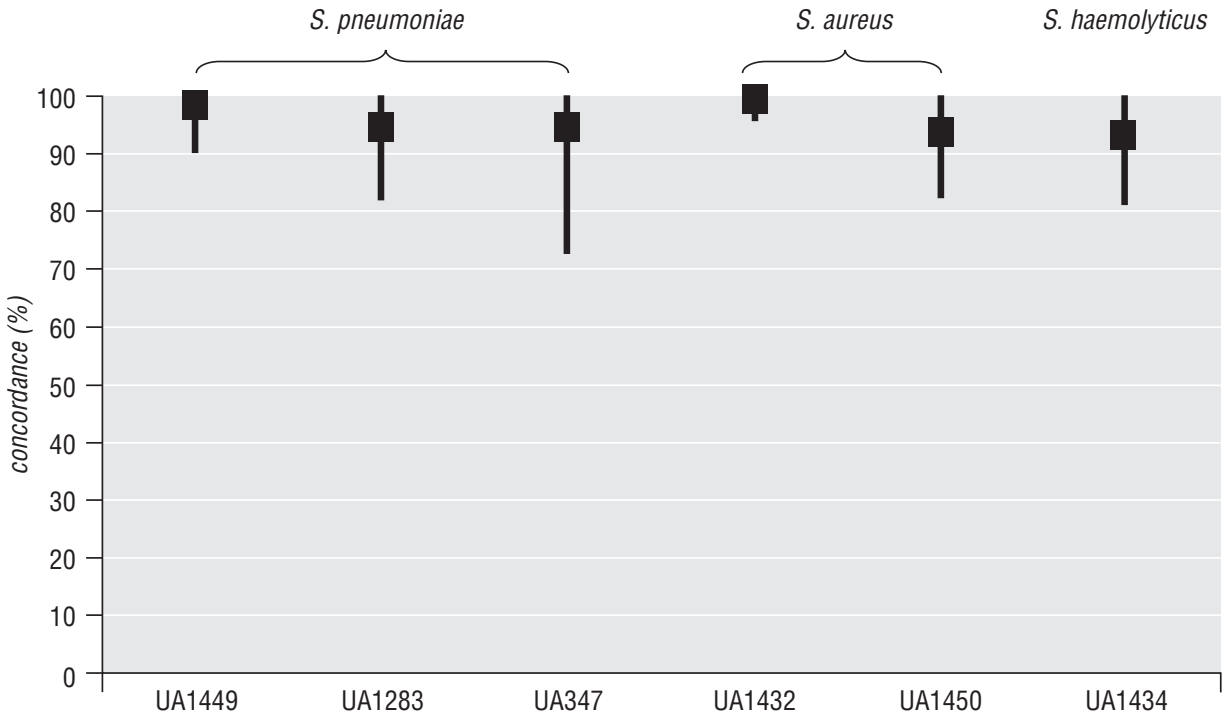

Figure 4.1. The average and the range across countries of the con cordance of antimicrobial test results, specified for every control strain.

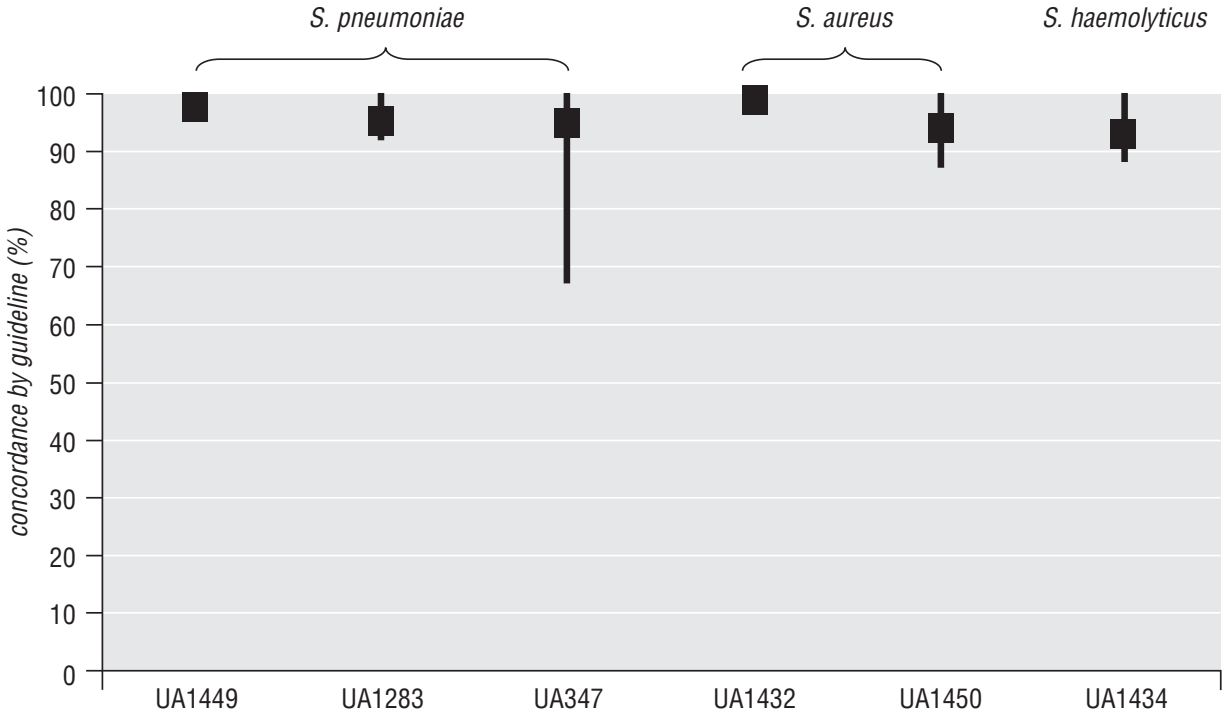

Figure 4.2. The average and the range across guidelines of the concordance of antimicrobial test results, specified for every control strain.

The proportion of laboratories that reported penicillin non-susceptibility correctly after testing penicillin G varied from 96% for strain UA1449 to 90% for strain UA1283 and 87% for strain UA347 (Table 4.4). The peak of the frequency distribution of the penicillin G MICs for strain UA1449 (2 mg/L), yielding the highest concordance is well in the 'non-susceptible' region compared with the peak of the frequency distribution of strain UA347 (0.25 mg/L), yielding a lower concordance. Again, almost all countries showed high concordance rates, with the exception of Bulgaria and Greece.
The overall concordance rate for the detection of penicillin G non-susceptibility among guidelines followed in Europe was 91%, as specified for all three *S. pneumoniae* strains and for every guideline in Table 4.5. Again, performance was best for strain UA1449 and decreased for the other two test strains.
Guidelines yielding somewhat lower concordance rates, such as those set by the Deutsches Institut für Normung (DIN), as well as the guidelines specified under 'Other' [Czech 98 and Mesa Española de Normalizacion de la Suseptibilitad y Resistencia a los Antimicrobianos (MENSURA)] were used by only a few participants. The guideline used most frequently for penicillin testing of *S. pneumoniae* was NCCLS, with an average concordance of 91%.
Erythromycin resistance in all three *S. pneumoniae* control strains was detected correctly by 99% of the participants.

Oxacillin, gentamicin, erythromycin, teicoplanin and vancomycin susceptibility in S. aureus. The overall concordance for detection of oxacillin (i.e. methicillin) resistance in the homogeneously resistant *S. aureus* strain UA1432 was 100%. The overall concordance for the heterogeneously resistant MRSA strain UA1450 was much lower, at 77%. Three countries (Czech Republic, Greece and Iceland) yielded notably lower concordance rates (Table 4.6), but no difference was found among different guidelines followed in Europe (data not shown).
We found a very high concordance for detection of vancomycin susceptibility in the two MRSA strains, of 98% and 100%, respectively.
Respectively 100% and 99% of the participants detected erythromycin and gentamicin resistance in the homogeneously resistant MRSA strain. Erythromycin and gentamicin susceptibility in the heterogeneously resistant MRSA strain was interpreted correctly by 98% and 99% of the participants, respectively. For teicoplanin susceptibility in the heterogeneously resistant MRSA strain, we found a concordance rate of 100%.

Teicoplanin, vancomycin, gentamicin and erythromycin susceptibility in S. haemolyticus. For detection of teicoplanin resistance in the *S. haemolyticus* strain, the overall concordance rate was 82% (Table 4.7), with five countries (Belgium, Denmark, Luxembourg, The Netherlands and UK) scoring low concordance rates. Vancomycin susceptibility of this strain was interpreted correctly by 94% of the participants.

Table 4.3. Detection of penicillin non-susceptibility in *S. pneumoniae* by country as tested with an oxacillin screen disc

	Strain UA1449 (oxacillin MIC of ref. labs: 16 mg/ L; intended interpretation: resistant)		**Strain UA1283 (oxacillin MIC of ref. labs: 2 mg/ L; intended interpretation: resistant)**		**Strain UA347 (oxacillin MIC of ref. labs: 4 mg/ L; intended interpretation: resistant)**		**Total**	
Country	**number of labs doing test**	**% correct**	**number of labs doing test**	**% correct**	**number of labs doing test**	**% correct**	**total of tests**	**% correct**
Austria	10	100	10	90	10	100	30	97
Belgium	51	100	50	98	48	100	149	99
Bulgaria	16	100	16	94	15	100	47	98
Czech R.	28	100	28	100	28	100	84	100
Denmark	5	100	4	100	5	100	14	100
Finland	21	100	21	95	20	95	62	97
Germany	30	100	30	87	30	97	90	94
Greece	12	83	12	75	12	67	36	75
Iceland	3	100	3	100	3	100	9	100
Ireland	17	100	17	100	17	100	51	100
Israel	3	100	3	100	3	100	9	100
Italy	43	98	42	95	41	95	126	96
Luxembourg	4	100	4	100	4	100	12	100
Netherlands	22	100	22	95	22	91	66	95
Poland	17	100	17	100	17	100	51	100
Portugal	15	100	15	100	15	100	45	100
Slovenia	7	100	7	100	7	100	21	100
Spain	23	100	23	96	22	100	68	99
Sweden	20	100	20	100	20	100	60	100
UK	21	100	21	100	20	100	62	100
Overall concordance		99		96		97		97
95% Confidence interval								94– 99

Table 4.4. Detection of penicillin non-susceptibility in *S. pneumoniae* by country after testing for penicillin G

	Strain UA1449 (penicillin MIC of ref. labs: 4 mg/ L; intended interpretation: resistant)		Strain UA1283 (penicillin MIC of ref. labs: 0.5 mg/ L; intended interpretation: intermediate)		Strain UA347 (penicillin MIC of ref. labs: 0.5 mg/ L; intended interpretation: intermediate)		Total	
Country	number of labs doing test	% correct	number of labs doing test	% correct	number of labs doing test	% correct	total of tests	% correct
Austria	8	100	8	100	8	88	24	96
Belgium	49	98	48	90	47	87	144	92
Bulgaria	11	82	11	64	11	55	33	67
Czech R.	28	100	27	93	27	93	82	95
Denmark	4	100	4	100	4	100	12	100
Finland	22	95	22	91	21	86	65	91
Germany	29	93	28	82	28	79	85	85
Greece	13	85	13	69	13	54	39	69
Iceland	2	100	2	100	2	100	6	100
Ireland	17	94	17	94	17	94	51	94
Israel	3	100	3	100	3	100	9	100
Italy	47	87	47	79	45	78	139	81
Luxembourg	5	100	5	100	5	100	15	100
Netherlands	24	100	23	96	24	83	71	93
Poland	17	100	17	100	17	100	51	100
Portugal	14	100	14	100	14	100	42	100
Slovenia	10	100	10	100	10	100	30	100
Spain	30	97	30	97	30	93	90	96
Sweden	20	100	21	100	21	100	62	100
UK	22	100	22	95	21	95	65	97
Overall concordance		96		90		87		91
95% Confidence interval								87– 95

Table 4.5. Detection of penicillin non-susceptibility in *S. pneumoniae* by guidelines used

	Strain UA1449 (penicillin MIC of ref. labs: 4 mg/ L; intended interpretation: resistant)		Strain UA1283 (penicillin MIC of ref. labs: 0.5 mg/ L; intended interpretation: intermediate)		Strain UA347 (penicillin MIC of ref. labs: 0.5 mg/ L; intended interpretation: intermediate)		Total	
Country	number of labs doing test	% correct	number of labs doing test	% correct	number of labs doing test	% correct	total of tests	% correct
BSAC	10	100	9	100	7	100	26	100
CRG	7	100	7	100	7	100	21	100
DIN	10	100	10	70	10	70	30	80
NCCLS	180	94	170	92	163	87	513	91
SRGA	13	100	14	100	13	100	40	100
Other	23	100	25	76	23	70	71	82
Not indicated	135	96	140	91	148	89	423	92
Overall concordance		96		90		87		91
95% Confidence interval								89– 93

'Other' = participants using NeoSensitabs or the Stokes method or following a different guideline or following more than one guideline. For abbreviations see footnotes to Table 4.9.

Gentamicin and erythromycin resistance were detected by 97% and 99% of participants, respectively. The overall concordance for detection of oxacillin susceptibility in the *S. haemolyticus* strain was 83%, with three countries (Bulgaria, Israel and Slovenia) clearly scoring lower.

Concordance of MIC yielding methods versus non-MIC methods. Of the 433 laboratories participating, 375 used methods yielding an MIC: 11 (3%) used agar dilution, 21 (6%) (micro-) broth, 202 (54%) Etest and 52 (14%) used exclusively an automated method. Eightynine laboratories used more than one method. One-quarter of all laboratories (110/433) made use of an automated method. The most frequently used system was one of the different generations of bioMérieux Vitek (Table 4.8). The 'MICs' determined by

Table 4.6. Detection of oxacillin non-susceptibility in *S. aureus* by country

	Strain UA1432 (oxacillin MIC of ref. labs: >256 mg/ L; intended interpretation: resistant)		**Strain UA1450 (oxacillin MIC of ref. labs: 8-64 mg/ L; intended interpretation: resistant)**		**Total**	
Country	**number of labs doing test**	**% correct**	**number of labs doing test**	**% correct**	**total of tests**	**% correct**
Austria	10	100	10	70	20	85
Belgium	55	100	56	73	111	86
Bulgaria	19	100	19	95	38	97
Czech R.	33	97	33	42	66	70
Denmark	4	100	4	75	8	88
Finland	26	100	26	81	52	87
Germany	28	100	28	86	56	93
Greece	16	100	16	63	32	81
Iceland	3	100	3	33	6	67
Ireland	10	100	10	80	20	90
Israel	3	100	3	100	6	100
Italy	48	100	47	79	95	89
Luxembourg	5	100	5	80	10	90
Netherlands	22	100	23	87	45	93
Poland	18	100	17	88	35	94
Portugal	15	100	14	71	29	86
Slovenia	10	100	10	90	20	95
Spain	29	100	29	90	58	95
Sweden	24	100	23	91	47	96
UK	10	100	10	70	20	85
Overall concordance		100		77		89
95% Confidence interval						85–92

automated systems should be considered as semi-quantitative data because only a very limited range of dilutions is used. However, in this study we group automated methods under the 'MIC yielding methods' as opposed to 'non-MIC methods'.

Table 4.7. Detection of oxacillin susceptibility and teicoplanin resistance in *S. haemolyticus* by country

	Strain UA1434 (oxacillin MIC of ref. labs: 0.5 mg/ L; intended interpretation: susceptible)		**Strain UA1434 (teicoplanin MIC of ref. labs: 32-64 mg/ L; intended interpretation: resistant)**	
Country	**number of labs doing test**	**% correct**	**number of labs doing test**	**% correct**
Austria	10	100	9	100
Belgium	55	84	43	65
Bulgaria	19	53	14	93
Czech R.	33	76	31	90
Denmark	4	75	2	50
Finland	26	81	13	77
Germany	28	100	28	96
Greece	16	81	14	79
Iceland	3	100	0	–
Ireland	10	90	17	71
Israel	2	50	2	100
Italy	46	83	49	90
Luxembourg	5	100	5	60
Netherlands	23	91	18	61
Poland	17	82	9	100
Portugal	15	93	12	83
Slovenia	10	50	8	100
Spain	30	93	28	89
Sweden	25	80	17	76
UK	10	90	20	65
Overall concordance		83		82

Quantitative methods yielding a penicillin G MIC [number of tests done (n) = 882] were more frequently concordant than non-MIC methods (n = 242) for *S. pneumoniae* strains (94% versus 79%). The same was true for detection of teicoplanin resistance in the *S. haemolyticus* strain, with a concordance of 91% for teicoplanin MIC methods (n = 182) versus 71% for non-MIC methods (n = 160).

For the two *S. aureus* strains, oxacillin MIC methods (n = 363) reached 99% concordance in the homogeneously resistant MRSA, and 76% concordance in the heterogeneously resistant MRSA. Other methods (n = 405) yielded a concordance of 100% and 78%, respectively.

Use of guidelines

Of the 395 laboratories specifying which guideline they used, 242 (61%) in 19 countries followed the NCCLS guideline (Table 4.9). Any other guideline was not followed by more than 6% of the laboratories, in at most two countries. With only one reference laboratory participating in France, and Norway not participating, the CA-SFM and Norwegian Working Group on Antibiotics (NWGA) guidelines were not represented.[5]

Thirty-eight of the 433 laboratories (9%) did not specify the guideline they followed.

Discussion

This Europewide QA exercise was characterized by an excellent response rate. It confirmed that an exercise of these dimensions is feasible and demonstrated the commitment of EARSS participants to quality. Strains were identified correctly at the genus and species level, and the average concordances over all control strains were high. We distributed strains that tested the laboratories' capability to identify the most clinically relevant resistances (penicillin G in *S. pneumoniae*, methicillin in *S. aureus* and glycopeptide in staphylococci) and feel reassured to continue using surveillance data generated by the participating national surveillance systems.

In this exercise, 8685 tests were reported and assessed but some 850 more results were expected. Laboratories were asked to test all antimicrobial agents listed on the report form, but in case they normally test for another agent from the same class they were asked to specify the name of this agent in the same box. This may have given rise to misunderstanding. Laboratories were asked furthermore to test the susceptibility using routine procedures. Apparently this was interpreted by a number of laboratories to test only those organism–antimicrobial combinations that they test routinely. Laboratories should be solicited in future QA exercises to test and report all the requested organism–antimicrobial combinations.

To screen for penicillin resistance in *S. pneumoniae*, almost all participants used the oxacillin 1 µg disc, achieving a very high concordance rate. This indicates that the oxacillin screen disc reliably discriminates susceptible from non-susceptible strains.

The concordance for penicillin resistance in *S. pneumoniae* is somewhat lower when laboratories test for penicillin G. This lower concordance rate is partly due to the fact that a substantial number of laboratories use non-MIC-based penicillin confirmation

techniques. Indeed, we observed a far higher concordance (94%) for quantitative methods yielding a penicillin MIC than for non-MIC-yielding methods (79%), confirming the rationale of the EARSS protocol to perform MIC determination on *S. pneumoniae* strains found to be non-susceptible by a screen test. The difficulty of laboratories using disc diffusion tests to recognize reduced penicillin susceptibility in *S. pneumoniae* has recently been described in another international QA survey.[6]

The differences in concordance among the three *S. pneumoniae* control strains are a reflection of how many dilution steps the penicillin MIC for the strain is distant from the susceptible breakpoint. Indeed, more laboratories misinterpreted strain UA347 as being penicillin susceptible than strain UA1449.

Some guidelines yielded lower concordance rates for the determination of penicillin resistance, like the DIN guideline as well as the guidelines specified under 'Other' in Table 4.5. For the DIN guideline, this may be related to the susceptible breakpoint, which is one dilution step higher. Almost all national guidelines in Europe, as well as the NCCLS guideline, consider isolates of *S. pneumoniae* to be non-susceptible to penicillin if the MIC is >0.06 mg/L.[7–11] The DIN guideline considers isolates to be non-susceptible to penicillin if the MIC is >0.12 mg/L.[12] However, it should be noted that the DIN, as well as the guidelines specified under 'Other', were used only by relatively small numbers of laboratories, allowing for larger variation. All three *S. pneumoniae* control strains were non-susceptible, and the high concordance rates represent a high sensitivity of EARSS laboratories to detect penicillin non-susceptibility in *S. pneumoniae*. It is not possible from this exercise to infer the specificity of EARSS laboratories to detect penicillin susceptibility in *S. pneumoniae*. Because all three *S. pneumoniae* strains were highly resistant to erythromycin, they were not really a challenge to participating laboratories. Virtually all laboratories correctly determined erythromycin resistance.

For the detection of oxacillin resistance in *S. aureus*, we included one strain that was homogeneously resistant and another strain that was heterogeneously resistant to oxacillin. Laboratories performed extremely well in detecting oxacillin resistance in the homogeneously MRSA strain, but the concordance rate dropped from 100% to 77% in the heterogeneously resistant MRSA strain. A notable proportion of laboratories in three countries failed to detect the heterogeneously resistant MRSA strain. However, although we observed differences in concordance among countries, we found no significant differences among guidelines. Detecting heterogeneously resistant MRSA possibly depends more on test methods used by individual laboratories than on differences in guidelines. Laboratories in most countries, and in some countries in particular, should scrutinize carefully their capability of detecting low-frequency resistant subpopulations, and ensure that proper laboratory methods are used to detect heterogeneously resistant MRSA strains.

Table 4.8. Manufacturer and type of automated system used by participating laboratories

Automatic method	Total	Automatic method	Total
BBL/BD Sceptor	14	Dade Behring Microscan	5
Becton Dickinson Pasco	5	Dade Behring Microscan Walkaway	11
bioMérieux ATP	2	Dade Behring Autoscan	2
bioMérieux Vitek1	6	Sensititre Aris	2
bioMérieux Vitek2	8	Soria Helgguipo Wider	8
bioMérieux Vitek32	6	Pasteur-Sanofi Pneumo PAC	1
bioMérieux Mini API	6	>1 method	6
bioMérieux API-ATB	5		
bioMérieux Vitek unspecified	23	**Total**	**110**

Detection of glycopeptide resistance in staphylococci is of paramount importance. For safety reasons we chose not to distribute a vancomycin intermediate (or resistant) strain among laboratories all over Europe, but instead distributed a *S. haemolyticus* strain that was resistant to teicoplanin. Vancomycin susceptibility of the two *S. aureus* control strains was interpreted correctly by participating laboratories, but teicoplanin resistance of the *S. haemolyticus* strain was often missed. Quantitative methods yielding an MIC were more frequently concordant than non-MIC methods for the detection of teicoplanin resistance against the *S. haemolyticus* strain (91% versus 71%).
Only a few participants misinterpreted gentamicin and erythromycin susceptibility in staphylococci, indicating that most participating laboratories are capable of determining gentamicin and erythromycin resistance.

This exercise provides a good overview of the guidelines being followed in Europe, with exception of the French and Norwegian guidelines. The NCCLS guideline is widely followed in Europe. In 10 countries NCCLS seems to be the only guideline in use; but in the countries that have issued national guidelines (Germany, The Netherlands, Sweden and Spain) some laboratories also follow the NCCLS guideline. The BSAC and Swedish Reference Group for Antibiotics (SRGA) guidelines are the only European guidelines used in more than one country. Because France and Norway are not represented in this study, we cannot infer on the use of guidelines there. It is probable, however, that the CA-SFM and NWGA guidelines are not widely followed in other European countries.
We found that 9% of participating laboratories did not specify which guideline they follow. Apart from the obvious reason that some laboratories simply may not have reported which guideline is followed, it may also be that some laboratories use in-house guidelines,

Table 4.9. The usage of guidelines by number of laboratories per country

Guideline used	BSAC	CRG	CZECH 98	DIN	MEN-SURA	NCCLS	CA-SFM[a]	SRGA	Stokes	>1	Not specified
Austria						9					1
Belgium						31				10	16
Bulgaria						20					
Czech R .			15			4				11	3
Denmark								1		1	3
Finland						25					
France							1				
Germany				15		6				9	1
Greece						14					3
Hungary						1					
Iceland						3					
Ireland	5					1			9	2	1
Israel						3					
Italy						50				2	1
Luxembourg						5					
Malta						1					
Netherlands		6				8				9	2
Poland						18			1		
Portugal						11				1	4
Slovenia						7			3		
Spain					1	25			5		
Sweden								25			
UK	12								6	3	3
Total	**17**	**6**	**15**	**15**	**1**	**242**	**1**	**26**	**15**	**57**	**38**
Grand total											**433**

[a]French laboratories did not participate in this QA exercise, with the exception of one national reference centre. BSAC, British Society for Antimicrobial Chemotherapy; CRG, Commissie Richtlijnen Gevoeligheidsbepalingen; DIN, Deutsches Institut für Normung; MENSURA, Mesa Española de Normalizacion de la Suseptibilitad y Resistencia a los Antimicrobianos; NCCLS, National Committee for Clinical Laboratory Standards; CA- SFM, Comité de l'Antibiogramme de la Société Française de Microbiologie; SRGA, Swedish Reference Group for Antibiotics; >1, more than one guideline followed.

or other non-documented guidelines. An overview of the antimicrobial susceptibility test breakpoints of national societies has been published recently.[13] The authors recommend that other national guidelines (e.g. Czech 98) are also documented in international literature and that every laboratory works according to well-documented guidelines so that susceptibility test results are reproducible and comparable. Guidelines should also be freely accessible through the Internet.

It should be noted that overall the breakpoints defining susceptibility or resistance of bacteria to antimicrobial agents do not differ greatly between guidelines used in Europe. Baquero[14] argued that it is possible to establish a theoretical consensus standard list of breakpoints, such that more than 95% of the breakpoints proposed by the different systems differ from the consensus standard by no more than one dilution.

We hope that these findings may add to the process of standardizing breakpoints in Europe as brought forward by the EUCAST.

It is shown that, overall, countries participating in EARSS are capable of delivering susceptibility data of good quality. The comparability of susceptibility data for penicillin resistance in *S. pneumoniae* and for homogeneous methicillin resistance in *S. aureus* is satisfactory among European countries and across guidelines. However, we emphasize the importance of determining an MIC for suspected penicillin non-susceptible *S. pneumoniae* and for suspected glycopeptide non-susceptible *S. aureus*.

Laboratories, particularly in some countries, may need to improve their capability of detecting oxacillin resistance in heterogeneously resistant MRSA and teicoplanin resistance in *S. haemolyticus*.

A number of laboratories did not fill out the form completely, for example by not reporting the species identification or not performing all the susceptibility tests requested. Not doing (or reporting) a test is considered as non-performance and hinders the assessment of the performance of laboratories. This should be avoided in future QA studies by organizers and participants.

Not every laboratory produced good results in this exercise, and the performance of some individual laboratories could probably be improved. For continuous external quality assessment we recommend that laboratories participate in national and international schemes with frequent distributions of control strains.

However, we feel reassured by this exercise that overall the antimicrobial susceptibility testing data as monitored through the national surveillance systems that participate in EARSS are of good quality. It is hoped that laboratories participating in this EARSS–UK NEQAS quality assurance are encouraged to maintain and improve their performance, as has been observed in other surveillance schemes.[15,16]

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References

1. EUCAST. (2000). The setting of antimicrobial breakpoints *Clinical Microbiology and Infection* **5,** 12.
2. EARSS website. (2001). EARSS Manual. EARSS management Team. [Online.] http://www.earss.rivm.nl (21 June 2002, date last accessed).
3. Tomasz, A., Nachman, S. & Leaf, H. (1991). Stable classes of phenotypic expression in methicillin-resistant clinical isolates of staphylococci. *Antimicrobial Agents and Chemotherapy* **35**, 124–9.
4. UK-NEQAS website. (2000). Report of External Quality Assessment Exercise EARSS-NEQAS 2000. UK-NEQAS. [Online.] http://www.pcug.co.uk/~ukneqasm/ and at http://www.earss.rivm.nl (21 June 2002, date last accessed).
5. Bergan, T., Bruun, J., Digranes, A., Lingaas, E., Melby, K. & Sander, J. (1997). Susceptibility testing of bacteria and fungi. Report from the Norwegian working group on antibiotics. *Scandinavian Journal of Infectious Diseases, Suppl. 1*, 103.
6. Tenover, F. C., Mohammed, M. J., Stelling, J., O'Brien, T. & Williams, R. (2001). Ability of laboratories to detect emerging anti-microbial resistance: proficiency testing and quality control results from the World Health Organization's external quality assurance system for antimicrobial susceptibility testing. *Journal of Clinical Microbiology* **39**, 241–50.

7. The British Society for Antimicrobial Chemotherapy (BSAC) website. (1998). Standardized disc sensitivity testing method, The Newsletter of the British Society for Antimicrobial Chemotherapy, 1998. BSAC, Birmingham, UK. Update available online at: http://www.bsac.org.uk/discdiff/sensitivity2.pdf (21 June 2002, date last accessed).
8. Commissie Richtlijnen Gevoeligheidsbepalingen. (1996). *Nederlands Tijdschrift voor Medische Microbiologie* **4**, 5.
9. Cars, O. (Ed.) (1997). Antimicrobial susceptibility testing in Sweden. *Scandinavian Journal of Infectious Diseases, Suppl. 1*, 105.
10. Société Française de Microbiologie. (1996). Report of the Comité de l'Antibiogramme de la Société Française de Microbiologie. *Clinical Microbiology and Infection* **2**, *Suppl. 1*, S1–49.
11. MENSURA. (2000). Recomendaciones del grupo MENSURA para la selección de antimicrobianos en el estudio de la sensibilidad y criterios para la interpretación del antibiograma. *Revista Española Quimoterapia* **13**, 73–86.
12. Deutsches Institut für Normung. (1998). Methods for the Determination of Susceptibility of Pathogens (Except Mycobacteria) to Antimicrobial Agents. *MIC Breakpoints of Antibacterial Agents, Suppl. 1,* pp. 58940–4. DIN, Berlin.
13. Degener, J. E. & Phillips, I. (2001). Comparison of antimicrobial susceptibility test breakpoints of national societies. *Clinical Microbiology and Infection* **7**, 51–4.
14. Baquero, F. (1990). European standards for antibiotic susceptibility testing: towards a theoretical consensus. *European Journal of Clinical Microbiology and Infectious Diseases* **7**, 492–5.
15. Forster, D. H., Krause, G., Gastmeier, P., Ebner, W., Rath, A., Wischnewski, N. et al. (2000). Can quality circles improve hospital-acquired infection control? *Journal of Hospital Infection* **45**, 302–10.
16. Snell, J. S. & Brown, D. F. J. (2001). External quality assessment of antimicrobial susceptibility testing in Europe. *Journal of Antimicrobial Chemotherapy* **47**, 801–10.